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Method for Concentration of Parasites from Small Amounts of Feces

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A total of 258 formalinized stool specimens received in our clinical laboratory were examined for parasites by direct smears and by the standard Formalin-ethyl acetate (FEAc) concentration method. Microconcentration (MC), a miniaturization of the FEAc method, was compared with the standard method for efficiency of parasite recovery. MC employed 0.25 to 0.50 ml of formalinized stools, 0.5 ml of Formalin, and 0.25 ml of ethyl acetate; the washing steps were omitted, whereas the rest of the procedure remained the same as the FEAc method. A total of 36 (13.9%) specimens were positive for parasites; of these, 23 (63.9%) were negative on direct examination. In 14 of these 23 specimens, the FEAc and MC methods were equivalent in detecting parasites. MC failed to detect parasites in eight specimens that were positive by FEAc and detected a parasite in one specimen that was negative by FEAc. Of 14 specimens positive by both concentration methods, FEAc detected additional parasite species in 2 specimens and MC did so in 1 specimen. The reduced sensitivity of parasite concentration evident in the MC we believe to be exclusively due to the drastically reduced sample size. We propose MC as an alternative to the FEAc concentration method when only small amounts of feces can be obtained.

The Formalin-ethyl acetate (FEAc) method for concentrating cysts of intestinal protozoa and ova of helminths is considered to be one of the most efficient methods for this purpose. One possible problem is that the procedure requires a minimum of 0.5 ml of washed feces. Often, as with some pediatric patients, this requirement cannot be met.

This is a study of microconcentration (MC), a modification of the FEAc method which can be performed with approximately 0.1 g of feces.

MATERIALS AND METHODS

Samples of stool varying from 0.09 to 0.17 g of feces were placed in 1-ml centrifuge tubes. Cryotubes, which are sterile, 1-ml capacity, polypropylene tubes with mounted screw caps, were obtained from Vanguard International, Inc., Neptune, N.J., and were used as centrifuge tubes. The specimens were not washed to avoid possible loss of parasites. By using a micropipetting device, 0.5 ml of 10% Formalin was added to the specimens. This achieved a range of Formalin to stool ratio of between 3:1 and 5:1. The specimens were stirred, and 0.25 ml of ethyl acetate was added to the mixture. The tubes were capped and shaken for 30 s. They were then centrifuged at 400 × *g* for 1 min in a model HN-S centrifuge equipped with a standard swinging bucket rotor (no. 958; International Equipment Co., Needham Heights, Mass.). The small tubes were placed directly into the carrier buckets and retrieved with forceps after centrifugation. Four layers resulted after centrifugation: excess ethyl acetate, a "cloudlike" layer of debris, Formalin, and the sediment. Owing to the nature of the debris layer, the supernatant may easily be decanted. Capillary tubes are suitable devices for drawing the sediment from the centrifuge tube and delivering it onto the slide. In most cases, only one wet mount (22 by 22 mm) could be prepared since only a small amount of sediment was obtained; this was stained with iodine (Lugol's, diluted 1:5).

A total of 258 formalinized stool specimens received in our laboratory were examined in three ways: direct wet mounts and wet mounts prepared from FEAc and MC concentrations.

Direct saline and iodine wet mounts were prepared from formalinized specimens and examined. FEAc concentrations were performed by the method described by Young et al. (3). Saline and iodine wet mounts were prepared from the concentrated sediment and examined.

A scoring system patterned after that of Young et al. (3) was used in comparing the quantity of each parasite species recovered in FEAc and MC concentrates versus direct examination. If an equal or smaller number of protozoan cysts was seen per ×400 field as compared to direct examination, the value given the concentrate for that particular parasite was zero. An improvement of 1 to 4 cysts per field was given a score of 1; 5 to 10, 2; 11 to 15, 3; >15, 4. Scores for helminth ova and larvae were based on improvement in the number of parasites seen per coverslip when scanning at ×100. Here again, if no improvement was noted over direct examination a score of zero was assigned.

An improvement of one to two ova or larvae per coverslip was given a score of 1; three to four, 2; four to five, 3; and greater than five, 4.

Microconcentration procedure. A total of 500 μl or 0.5 ml of 10% Formalin was added to the specimen, which was then stirred with applicator sticks. Then, 250 μl or 0.25 ml of ethyl acetate was added, the cap was replaced, and the tube was shaken for 30 s and centrifuged at 400 × *g* for 1 min. The cap was removed, and all of the supernatant was decanted. The tip of a capillary tube was placed into the sediment and the mixture was allowed to draw by capillary action. The concentrate was expelled onto the slide by tapping the tip of the capillary tube on the slide, 1 drop of iodine and a coverslip (22 by 22 mm) were added, and the slide was sealed with molten petroleum jelly-paraffin mixed in approximately a 50:50 proportion.

RESULTS

Of 258 specimens, 36 were positive for parasites; of these, 23 (63.9%) were negative and 13 (36.1%) were positive by direct smear. In 14 of the 23 specimens negative by direct examination, the FEAc and MC methods were equivalent in concentration of parasites. Within this group of 23 specimens, MC did not concentrate parasites that were recovered by FEAc in 8 specimens, and conversely, FEAc did not concentrate a parasite recovered by MC from one specimen (Table 1). Of the 13 specimens positive by direct examination, parasites were detected by both MC and FEAc in 12, and one specimen was positive only by MC (Table 2). Of 26 specimens positive by both concentration methods, regardless of direct examination result, FEAc detected additional parasite species in five specimens and MC did so in one specimen.

The 36 positive specimens contained a total of 46 species of parasites. The scoring system allowed each concentration procedure to be evaluated for each parasite species individually (Table 3). The mean score for FEAc was 2.04, whereas the mean score for MC was 1.65. These data were analyzed by nonparametric one-way analysis of variance. The difference in recovery rates was found to be insignificant at a 5% probability level.

DISCUSSION

The MC procedure was developed to improve the rate of recovery of parasites when only small amounts of feces can be obtained. A variety of situations may arise that will make difficult or prevent obtaining a stool sample adequate for a standard-volume concentration method. In our institution, we see a number of naturally or induced immunodeficient children, particularly neonates, in whom intestinal parasites are suspected. A specimen adequate for the standard FEAc method is usually not obtainable in these children. In addition, the MC method may be useful when a specimen container leaks in transit to the laboratory and replacement of the specimen is difficult or delayed. In the veterinary or animal research setting, MC may be employed to examine single stool passages from small animals that would not produce sufficient specimen for a standard-volume method. Lastly, the method may be useful in field surveys of human or animal populations where, for logistical or other reasons, only small samples are available. Our search for a method to concentrate parasites from minute specimens was initiated in response to such a situation (1). A parasitological survey of a remote area of the world was accomplished as part of a medical expedition. However, because access was solely by several days of travel on foot, only very small specimens could be returned to our laboratory in keeping with weight restrictions.

TABLE 1. Comparison of concentration methods in specimens with negative direct smears (% of total)

FEAc result	MC result		Total
	MC positive	MC negative	
FEAc positive	14	8	22 (95.6)
FEAc negative	1	0	1
Total	15 (65)	8	23 (100)

A limitation of the MC procedure is the greatly reduced sample employed, since it is logical to expect that there will be specimens in which the load of parasites will be sufficiently small for sample size to be a critical factor in the detection of parasites by any concentration procedure. The sample recommended for the standard Formalin-ether method of Ritchie (2), as well as for the FEAc method, is approximately 1 g of stool. The MC method employs approximately 0.1 g of stool or between 0.25 to 0.5 ml of formalinized feces (assuming a 3:1 ratio of Formalin to feces). With only a few parasites in a specimen, such a drastic reduction in sampling increases the probability of including no parasites in a given sample; obviously, the concentration efficiency of a method then becomes irrelevant.

Table 1 demonstrates a comparison of both concentration methods with specimens that

TABLE 2. Comparison of concentration methods in specimens with positive direct smears (% of total)

FEAc result	MC result		Total
	MC positive	MC negative	
FEAc positive	12	0	12 (92.3)
FEAc negative	1	0	1
Total	13 (100)	0	13 (100)

TABLE 3. Comparison of concentration methods by parasite

Parasite	Avg score		No. of specimens
	FEAc	MC	
Giardia lamblia	1.9	1.8	12
Endolimax nana	1.0	2.0	2
Clonorchis sinensis	2.4	2.1	17
Strongyloides stercoralis	0.5	0.5	2
Hookworm	1.3	0.6	6
Entamoeba coli	2.9	2.3	4
Taenia sp.	2.5	0	2
Trichuris trichiura	2.0	0	1

were negative by direct examination. Since these specimens were negative by direct examination, the assumption may be made that there is a low number of parasites present. Thus, FEAc is more efficient in detecting parasites in this group of specimens (Table 1). We feel that this is due solely to the 10-fold larger sample used in the FEAc method. In Table 2, the same comparison is made with specimens that were positive by direct examination and thus contain a heavier parasite density. In this group, the methods are equivalent, in fact, MC detected one positive that was missed by FEAc concentration. These data point out that the critical component distinguishing these two methods is the size of the sample. If the MC method is employed, we should be mindful of the fact that, of positive specimens that were negative by direct examination alone, MC detected only 65% of those detected by the standard-volume FEAc method. Conversely, all 13 specimens positive by direct examination were positive by MC as well, whereas FEAc missed 1 of these. Of the 36 positives, MC detected 28 (77.8%), whereas FEAc detected 34 (94.4%). Therefore, the recommendation can be made that, with minute stool samples, the MC be performed with the entire sample, without need for direct examination.

We propose MC as an alternative to the FEAc concentration method when only small amounts of feces can be obtained. Given the limitation imposed by the reduced sample, we suggest that the MC method not be used routinely but only when dictated by the situation.

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